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INHERITANCE OF BLAST DISEASE (*Magnaporthe grisea*) RESISTANCE IN *INDICA* RICE (*Oryza sativa* L.) CV. HUR 4-3, TETEP AND THEIR SEGREGATING GENERATIONS

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ABSTRACT

The fungus *Magnaporthe grisea* (causes blast disease) isolate *LB-TN-2* was used to study the genetics of blast disease resistance in *indica* rice cultivars. Six generations (P_1, P_2, F_1, F_2, B_1 and B_2) of cross HUR 4-3 × Tetep were utilized for the study of blast disease inheritance. The *indica* rice cultivar 'Tetep' showed 10.29 % disease severity (DS) against *Magnaporthe grisea* isolate *LB-TN-2* and classified as resistant cultivar, while high yielding, early maturing cultivar 'HUR 4-3' showed 41.13 % disease severity and classified as susceptible cultivar. The area under the disease progress curve (AUDPC) of resistance cultivar was observed 98.78 which are significantly less than the susceptible cultivar 364.18. The F_1 (HUR 4-3 × Tetep) plants were observed to be resistant with average DS and AUDPC are 12.47% and 135, respectively. The F_2 populations were observed to show three distinct phenotypic classes; resistant, moderately resistant and highly susceptible with a ratio of 9:6:1, respectively. Two backcross populations, B_1 and B_2 showed different response from each other during pathogen inoculation, evaluation which results in the phenotypic ratio of 1-R: 2-MR: 1-S in B_1 and 1-R: 0-S in B_2, respectively. The result revealed that the blast disease resistance against fungal pathogen *Magnaporthe grisea* virulent isolates LB-TN-1 due to polymeric gene action or duplicate cumulative effects of two dominant major resistant genes *i.e.*, *Pi1* and *Pi54* with synergistic effects of other related minor genes.

KEY WORDS: Blast resistance, disease intensity and severity, inheritance, polymeric gene action, *indica* rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is the second most important cereal crop in the world after wheat and feeding over half of the world population. The 90% of world rice produces and consumed in Asian countries (Khush, 2005; Singh *et al*., 2013a; Verma *et al*., 2017 and Singh *et al*., 2019a). The rice crop can be grown in diverse ecological condition like; rainfed low land, rain fed upland and flood prone /deep water environment due to its wide range of adaptability and hardiness for different agro-climatic zone (Khush, 2005; Khush, 2013; Singh *et al*., 2013b, c and Singh *et al*., 2014a, b). In Asia, rice coverage an area of 137m ha for its cultivation wherein India has a major share of 44.6 m ha (23.3% of gross cropped area of the country) with the production of 115.6 m t (next to China, 141.6 m t) and average productivity of 2.59 t/ha (FAO STAT, 2018; FAO RMM, 2019 and Singh *et al*., 2020). With such diverse growing area of rice is also prone to 70 different types of diseases caused by several biotic agents *i.e*., fungi, bacteria, nematode and viruses causing constitutively 5.5 to 29.0% yield loss every year (Song and Goodman, 2001; Singh *et al*., 2013b, c and Singh *et al*., 2020). Among these diseases, rice blast caused by the fungal pathogen *Magnaporthe grisea* reported as an overwhelming restriction to rice production occurring in more than 85 rice growing countries globally (Scardaci *et al*., 1997; Gilbert *et al*., 2004; Singh *et al*., 2013b and Singh *et al*., 2020). The fungus *Magnaporthe grisea* is a hemi biotrophic, heterothallic, ascomycetous fungus which potentially can occur in all stage of growth and causing heavy and total loss (Sharma *et al*., 2012 and Singh *et al*., 2013a, b, c). More than 130 blast resistance genes have been described and mapped by preceding workers (Singh *et al*., 2020) but a partial number of reports are obtained on the genetics of blast resistance in rice in rice varieties (Sharma *et al*., 2012). Meanwhile, the dynamic changes in the race composition of pathogen has often caused breakdown of resistance in most of the improved resistant varieties. In rice varieties, the blast disease resistance is mostly governed by dominant or major genes, but in few cases, recessive genes are also responsible for resistance (Singh *et al*., 2013b, c; Singh *et al*., 2019a and Singh *et*

al., 2020). Elite cultivars containing a single major resistance gene become susceptible within few years. Stacking of more than one major resistance gene has been proven one of the effective methods to deliver durable resistance against rice blast (Hittalmani *et al*., 2000 and Singh *et al*., 2018). For breeding durable rice blast resistance, the knowledge of inheritance pattern of blast disease is prerequisite.

Keeping all above facts in mind, an attempt has been taken to study the inheritance of various kind of genic effects of blast disease under artificial inoculation for blast pathogen in the field condition by using six generations (P_1, P_2, F_1, F_2, B_1 and B_2) of a cross of blast disease susceptible and resistant cultivar. The information about the nature and magnitude of gene action or genic inheritance existing in the breeding material would be a valuable tool for selecting appropriate breeding system and hence to achieve the preferred genetic enhancement in stress breeding.

MATERIALS AND METHODS

The investigation was conducted during *Kharif* season in 2014-15 and 2015-16 at the experimental form of Department of Genetics and Plant Breeding Institute of Agricultural Sciences, Banaras Hindu University, Varanasi and *Off*-season (*Rabi* season) in 2014-15 at Indian Council of Agricultural Research (ICAR) -National Rice Research Institute (NRRI), Cuttack, Odisha. The experimental material for this study is two *indica* rice cultivars HUR4-3 (high yielding, semi dwarf, medium maturing, fine with acceptable grain quality but susceptible to Blast disease cultivar) and Tetep (Blast resistant cultivar, carrying resistant genes *Pi1* and *Pi54*), used as recurrent and donor parents, respectively. Both the parents (HUR 4-3 and Tetep) were timely sown in the experimental field at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (Uttar Pradesh) in two different dates for flowering synchronization during *Kharif* -2014. Crosses among parents (HUR 4-3 and Tetep) were made to produce F_1 hybrid seeds. These F_1 seeds along with both parents were planted at ICAR-National Rice Research Institute, Cuttack (Odisha) during *Off*-season/ *Rabi* in 2014-15. The 20-25 plants of true F_1's hybrids were backcrossed with both parents to generate the backcross progenies *i.e.*, B_1 (F_1 × HUR4-3) and B_2 (F_1 × Tetep) generations and the remaining 25-30 F_1's plants were selfed to produce the seeds of F_2 populations. The seedlings of parents (HUR 4-3:P_1 and Tetep:P_2) along with four segregating generations (F_1, F_2, B_1 and B_2) and blast disease susceptible check Co 39 were transplanted in a complete family randomized block design with three replications. The plant populations were maintained with a spacing of 15 × 20 cm plant to plant and row to row, respectively in the experimental field of Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (Uttar Pradesh) during *Kharif* 2016. All the recommended cultural practices were applied to grow healthy crop excluding the blast disease control.

The virulent isolate *i.e*., *LB-TN-2* of fungus *Magnaporthe grisea* were obtained from the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi from the blast disease infected leaf of rice plants. Isolation of fungus was carried-out under aseptic conditions by spore-drop method following the protocol describe in Rajashekara *et al*., 2016. The isolate was cultured on potato dextrose agar (PDA) and Oat Meal Agar (OMA) medium in petri plates and incubated at 28^{o}C. The morphological identification confirmed the characteristics of pathogen *Magnaporthe grisea i.e.,* pyriform to oblong conidia which are hyaline in colour and bi-septate measuring 19 - 27 × 8 - 10 µm in size. The disease screening plots/ field of both the parents (HUR 4-3 and Tetep) as well as segregating populations *i.e*., F_1, F_2, B_1 and B_2 were inoculated by spray of 15 days old culture obtained from OMA media at a concentration of 1×10^5 conidia per ml and solution also contain tween-20 (0.2 %). The inoculated plants were observed thrice in seven days interval *i.e.,* 7, 14 and 21 days after inoculation (DAI). The disease scoring was performed using 0-9 scale of standard evaluation system of IRRI-SES scale as described in table 1 of SES, IRRI, 1996, 2013 and Singh *et al.*, 2013c and data were recorded.

The data on disease screening or scoring were calculated for disease severity percent (DSP) and area under disease progress curve (AUDPC) according to the formulae described by Sabin *et al*., 2016 and Singh *et al*., 2018. The plants were categorized as resistance and susceptible for rice leaf blast based on their disease scores and disease severity. These observed frequencies were further tested using $\chi 2$ test for goodness-of-fit with expected frequencies of resistant and susceptible plants to study the pattern of inheritance of blast resistance in rice following the Mather and Jinks, 1971 and Singh *et al*., 2014b.

RESULTS AND DISCUSSION

The *indica* cultivar used in present investigation 'Tetep' was displayed resistant disease reaction against fungus *Magnaporthe grisea* under epiphytotic condition using artificial inoculation of isolate of *LB-TN-2* in the field condition due to presence of two major dominant resistance genes *Pi1* and *Pi54* and other minor genes which showed disease score 1 with 10.29 per cent disease severity. While, the other high yielding cultivar 'HUR4-3' displayed susceptible reaction with disease score 7 and per cent disease severity was 41.13 % due to absence of these two or other resistant genes (Table 2). The initial symptoms of blast disease were observed on the high yielding cultivar 'HUR4-3' with variable intensities in the form of gray green and water-soaked lesions with a darker green border, which extended rapidly to few centimeters in length, and further converted into typical diamond shaped lesions of blast disease.

TABLE 1: Scale for scoring of rice leaf blast disease (IRRI, 2013, Singh *et al*. 2013c)

Scale	Disease severity	Host response
0	Lesion are not present	Resistant (R)
1	Small brown specks of pin point size or larger brown specks without sporulating center	Resistant (R)
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves	Resistant (R)
3	Lesions type is same as in scale 2, but a significant number of lesions on upper leaf area	Resistant (R)
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4 % of leaf area	Moderately Resistant (MR)
5	Typical susceptible blast lesions infecting 4-10% of the leaf area	Moderately Resistant (MR)
6	Typical susceptible blast lesions infecting 11 – 25% of the leaf area	Moderately Susceptible (S)
7	Typical susceptible blast lesions infecting 26 - 50% of the leaf area	Susceptible (S)
8	Typical susceptible blast lesions infecting 51-75% of the leaf area and many leaves are dead	Susceptible (S)
9	More than 75% leaf area affected	Susceptible (S)

These results are in agreement with earlier findings for symptoms on susceptible cultivars (Namrata *et al.*, 2019; Singh *et al*., 2019a, b and Singh *et al*., 2020). The area under the disease progress curve (AUDPC) of resistant cultivar was found 98.78 which is significantly lower than the susceptible recipient parent i.e., 364.18. The above findings are in accordance with earlier reports of wide difference between AUDPC of resistant and susceptible cultivars (Mohapatra *et al*., 2008; Singh *et al*., 2014a, b; Nguyen *et al*., 2015; Singh *et al*., 2018 and Namrata *et al.*, 2019). All the F_1 plant of cross HUR 4-3 × Tetep were observed to be resistant to moderately resistant when screened with a virulent isolate of blast disease i.e., *LB-TN-2* with average disease severity 12.47 % and AUDPC 135.07. These findings are in the good agreement of the earlier reports on resistant response of F_1 generation in cross of susceptible and resistant cultivars (Gupta *et al*., 2012; Singh *et al*., 2013b, c and Namrata *et al*., 2019).

Plants from F_2 generation were scored individually and could be classified into four distinct genotypic classes in a ratio of 9:3:3:1 and further re-classified as 9 : 6 : 1 ratio based on the phenotypic responses or disease reaction viz., resistant, moderately resistant and susceptible reaction against virulent isolate of blast disease *i.e*., *LB-TN-2* during investigation (table 2 and 3). The average lesion numbers in F_2 populations were recorded as 9.83 % to 52.46 with 124.97 to 550.59 AUDPC value and 12.40 to 51.28 per cent disease intensity (PDI), respectively. Among the evaluated 320 F_2 segregating plants, 171 plants showed resistant response, 125 plants showed medium/ moderately resistant response and 24 plants showed highly susceptible reaction against blast disease in the ratio of 9 : 6 : 1 with χ^2= 1.46, P > 0.05 revealed that observed data are in accordance with expected ratio. These results confirmed the modification of mendelian dihybrid ratio 9:3:3:1 into 9:6:1 ratio which was due to presence of two dominant and other minor related genes exhibited polymeric gene action or we can say duplicate genes with cumulative effect. These findings are in contradiction with reports of single dominant gene governing blast resistance in rice (Fuji and Saito, 2007; Sharma *et al.*, 2007 and Ashkani *et al.*, 2011) and partial agreement with the earlier reports of two dominant genes showing interaction for governing blast resistance in rice (Filippi and Prabhu, 1996; Persuad *et al.*, 2007; Zewdu *et al.*, 2018 and Namrata *et al*., 2019).

Two backcross generations, B_1 (F_1 × HUR4-3) and B_2 (F_1 × Tetep) of the cross showed different response from each other during evaluation for blast disease resistance using virulent isolate *LB-TN-2*. These findings show good amount of similarity with earlier reports (Persuad *et al*., 2007; Singh *et al*., 2014 a,b and Singh *et al*., 2018). The plants from B_1 generation showed three types of responses which included resistant, medium resistant and highly susceptible response. Average lesion number per plant showed by B_1 generation varied from 18.43 to 38.56 with 210.49 to 360.90 AUDPC value and per cent disease severity varied from 20.55 to 38.50 observed.

TABLE 2: Comparison of per cent disease severity or incidence (PDI), Area Under Disease Progress Curve (AUDPC) in six generations/ progenies (P_1, P_2, F_1, F_2, B_1 and B_2) of cross HUR 4-3 × Tetep against *Magnaporthe grisea* isolate *LB-TN-2*

Six generation with population size	Plant classified in resistant: susceptible group	Percent disease incidence (PDI) at 7 days interval of inoculum			AUDPC value	Disease severity (21 DAI)	Lesion number per plant	Disease score (21 DAI)	Host response on disease reaction (21 DAI)
		7 DAI ± SD	14 DAI ±SD	21 DAI ± SD					
Check (Co-39) 60 plants	60 HS	24.91 ± 1.56	40.56 ± 2.40	51.67 ± 1.64	551.97	51.67 ± 1.64	53.67	9	HS
P_1 (HUR 4-3) 60 plants	60 S	12.05 ± 1.36	25.44 ± 0.82	41.13 ± 1.03	364.18	41.13 ± 1.03	43.37	7	S
P_2 (Tetep) 60 plant	60 R	3.50 ± 0.49	7.22 ± 0.36	10.29 ± 0.95	98.78	10.29 ± 0.95	5.76	1	R
F_1's hybrid 60 plants	56R : 4MR	6.62 ± 0.65	9.75 ± 0.69	12.47 ± 1.16	135.07	12.47 ± 1.16	8.97	1	R/ MR
F_2's population 320 plants	171 R	5.26 ± 0.38	9.03 ± 0.34	12.40 ± 0.30	124.97	12.40 ± 0.30	9.83	1	R
	125 MR	12.46 ± 1.06	18.43 ± 0.89	23.18 ± 0.64	253.70	23.18 ± 0.64	19.43	5	MR
	24 HS	25.42 ± 1.29	40.31 ± 1.93	51.28 ± 1.07	550.59	51.28 ± 1.07	52.46	9	S/ HS
B_1 (F_1 × HUR 4-3) 120 plants	26 R	8.98 ± 0.39	15.20 ± 0.71	20.55 ± 0.90	210.49	20.55 ± 0.90	18.43	1	R
	67 MR	10.42 ± 0.78	22.46 ± 0.61	28.53 ± 0.88	293.57	28.53 ± 0.88	24.67	3	MR
	27 HS	14.37 ± 1.39	25.12 ± 1.54	38.50 ± 1.08	360.90	38.50 ± 1.08	38.56	7	S
B_2 (F_1 × Tetep) 120 plants	56 R	5.42 ± 0.64	9.69 ± 0.71	14.64 ± 0.57	138.06	14.64 ± 0.57	9.36	1	R
	64 MR	11.42 ± 2.11	16.28 ± 3.26	26.42 ± 2.73	246.40	26.42 ± 2.73	23.67	3	MR

Whereas, PDI: Percent disease intensity/ incidence, DAI: Days after inoculation, SD: Standard deviation, AUDPC: Area under disease progress curve, HS: Highly susceptible, S: Susceptible, R: Resistant and MR: Moderately resistant

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TABLE 3: Inheritance of blast disease resistance in six generations/ progenies (P_1, P_2, F_1, F_2, B_1 and B_2) of *indica* rice cross HUR 4-3 × Tetep against *Magnaporthe grisea* isolate LB-TN-2

Six generation with population size	PDI (21 DAI) with SD (%)	AUDPC value	Host response on disease response (21 DAI)	Gene combination expected	No. of plant observed	Geno-typic ratio	No. of plant expected	Chi-square value of population	Phenotypic ratio	Chi-square value of ratio
Co-39 (Check) 60 plants	51.67 ± 1.64	551.97	HS	*pi54 pi54pi1 pi1*	60	-	60	NS	-	NS
P_1 (HUR 4-3) 60 plants	41.13 ± 1.03	364.18	S	*pi54pi54pi1pi1*	60	-	60	NS	-	NS
P_2 (Tetep) 60 plant	10.29 ± 0.95	98.78	R	*Pi54Pi54Pi1Pi1*	60	-	60	NS	-	NS
F_1's hybrid 60 plants	12.47 ± 1.16	135.07	R/ MR	*Pi54pi54Pi1pi1*	56	-	60	NS	-	NS
F_2's population 320 plants	12.40 ± 0.30	124.97	R	*Pi54_Pi1_*	171	9	180	0.45	9 : 6 : 1 (171 : 126 : 23) R : MR : HS	1.46
	23.18 ± 0.64	253.70	MR	*Pi54_ pi1pi1: pi54pi54 Pi1_*	125	6	120	0.21		
	51.28 ± 1.07	550.59	HS	*pi54pi54pi1pi1*	24	1	20	0.80		
B_1 (F_1 × HUR 4-3) 120 plants	20.55 ± 0.90	210.49	R	*Pi54pi54 Pi1Pi1*	26	1	30	0.53	1 : 2 : 1 (26:67:27) R : MR : S	1.65
	28.53 ± 0.88	293.57	MR	*Pi54pi54pi1pi1: pi54pi54Pi1pi1*	67	2	60	0.82		
	38.50 ± 1.08	360.90	S	*pi54pi54, pi1pi1*	27	1	30	0.30		
B_2 (F_1 × Tetep) 120 plants	14.64 ± 0.57	138.06	R	*Pi54Pi54Pi1Pi1: Pi54Pi54Pi1pi1*	56	1:1	60	NS	1 : 0 All resistant	NS
	26.42 ± 2.73	246.40	MR	*Pi54pi54 Pi1Pi1: Pi54pi54Pi1pi1*	64	1:1	60			

Whereas, PDI: Percent disease intensity, DAI: Days after inoculation, SD: Standard deviation, AUDPC: Area under disease progress curve, DR: Disease reaction, HS: Highly susceptible, S: Susceptible, R: Resistant; MR: Moderately resistant and NS: Non-significant

Out of 120 plants were observed in B_1 generation, 26 plants showed resistant response, 67 plant moderately resistant and 27 plants highly susceptible response with $\chi 2 = 1.65$, $P > 0.05$ indicating that observed data are in agreement with the expected ratio in backcross generation and confirm modification of Mendelian dihybrid ratio of 1:1:1:1 into 1:2:1 ratio. However, in B_2 generation, 120 plants were observed and all the plants showed resistant response, hence, $\chi 2$ test was not applicable here due to presence of only class of resistance (degree of freedom= n-1) with $\chi 2 = 0$, $P > 0.05$ which revealed the modification of Mendelian dihybrid ratio of 1:1:1:1 into 1:0 ratio. Plants in B_2 generation was observed having average lesion number per plant as 9.36 to 23.67, AUDPC value varied from 138.06 to 246.40 and per cent disease incidence from 14.64% to 26.42%, respectively (table 2 and 3). These results showed that blast disease resistance in two backcross generations was governed by two dominant genes (*Pi1* and *Pi54*) with synergistic effect of other minor related resistant genes which showed polymeric gene action. Blast disease resistance is governed by two dominant genes (*Pi1* and *Pi54*) in interaction was earlier reported but they reported presence of two independent dominant genes or complementary gene interaction (Persuad *et al.*, 2007 and Zewdu *et al.*, 2018), inhibitory gene interaction (Singh *et al*., 2014a, b; Singh *et al*., 2018 and Kumar *et al*., 2019).

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